CLINICAL RESEARCH

Sural nerve oxygen tension in diabetes

P G NEWRICK, A J WILSON, J JAKUBOWSKI, A J M BOULTON, J D WARD

Abstract

Peripheral nerve oxygen tensions were assessed in vivo by using microelectrodes to measure endoneurial oxygen tension in exposed sural nerve. In 11 diabetic patients with chronic sensorimotor neuropathy the mean endoneurial oxygen tension was 39·7 (SD 10·2) mm Hg. In all but one patient compared with none of four non-neuropathic subjects the mean nerve oxygen tensions were below dorsal foot vein values.

This unphysiological state may have a role in the aetiology of diabetic neuropathy.

Introduction

The aetiology of the diabetic peripheral neuropathies remains unclear. Metabolic abnormalities in diabetic nerve are well described,[1] though the relation between biochemical abnormalities and various measures of severity of neuropathy in man is not clear cut.[2] The role of small vessel disease, proposed by Woltman and Wilder in 1929[3] and later by Fagerberg,[4] is also poorly understood. Sural nerve biopsies in diabetics with severe progressive neuropathy despite good blood glucose control have shown small vessel disease within the nerve.[5] Capillary closure and endothelial hyperplasia are significantly more common in diabetics with neuropathy compared with non-diabetics and diabetics without neuropathy.[6] Recent work in animals suggests that there is both ischaemia and hypoxia in diabetic nerve,[7] but this has not been shown in man. Oxygen supplementation ameliorates abnormalities of neurophysiology and nerve biochemistry in rats with diabetic neuropathy.[8] Conversely, normal rats kept in a state of chronic hypoxia develop impairment of nerve conduction velocity and abnormal ischaemic conduction block.[8] It is therefore important to know if human diabetic nerve is hypoxic, as this will have implications for research into the aetiology of diabetic neuropathy. We report, for the first time, a technique for measuring peripheral nerve oxygen tensions in man.

Royal Hallamshire Hospital, Sheffield S10 2JF
P G NEWRICK, MB, MRCP, Pfizer diabetes research fellow
A J WILSON, PHD, medical physicist
J JAKUBOWSKI, FRCS, consultant neurosurgeon
A J M BOULTON, MD, MRCP, medical senior registrar
J D WARD, MD, FRCP, consultant physician

Correspondence to: Dr Newrick, floor P.

Patients and methods

Platinum microelectrodes were employed which work on a polarographic principle.[9] When such an electrode is polarised negatively with respect to a silver-silver chloride electrode and the polarising voltage increased there is an initial rapid rise in current followed by a plateau with little increase in current for an increase in polarising voltage (typically 400-800 mV). Thereafter there is a further increase in current with polarising voltage. Within the plateau region the measured current is proportional to the oxygen tension within the fluid surrounding the electrode tip.

Electrodes were constructed by soldering a 3 cm length of 25 μm wire of 90% platinum and 10% iridium (Goodfellow Metals Ltd, Cambridge Science Park) to a 14 cm length of 90 μm copper wire. This was glass coated using a double pull technique with 2 mm diameter glass electrode tubes (Jencons Scientific Ltd, Leighton Buzzard) in an electrode puller (Harvard model 50-2047) and the open end sealed with wax. The tip was exposed by insertion into molten solder glass (Corning 7530) and withdrawn when solidified, thus exposing the wire in a controlled fashion. The tip was fire polished, enough to melt the glass to give a gas tight seal. The tip was then etched with three volts of alternating current in 25% sodium nitrite and dip coated with a semipermeable membrane of DPX mounting medium, giving a conical tip of about 5 μm diameter at the base and 10 μm long. Calibration was performed for 0% and 21% oxygen and electrodes not showing satisfactory polarographic characteristics rejected. Electrodes were sterilised by γ irradiation (3 Mrads). The electrodes were unpacked in the theatre and the tips rehydrated in isotonic saline for four hours on the day of the procedure.

Volunteers from the diabetic clinic were fully assessed (by PGN) to identify those with definite neuropathy. Assessment included electrophysiology with a Medelec DF06 (Medelec Ltd, Woking) and measurements of vibration perception threshold with a biothesiometer (Biomedical Instrument Co, Ohio) and thermal discrimination threshold with a thermoaesthesiometer (medical instrument department, Free University Hospital, Amsterdam). Minimum criteria for neuropathy were: (*a*) clear cut neuropathic symptoms of at least six months' duration; (*b*) abnormal sensory threshold (vibration perception or thermal discrimination >3 SD from the mean) or a peroneal motor nerve conduction velocity <40 m/s; and (*c*) absence of large vessel disease.

Patients giving informed consent and who were undergoing sural nerve biopsy proceeded as follows. Blood was taken from a dorsal foot vein, without compression and with the patient lying down, for measurement of venous oxygen. Patients were not fasted for the procedure and took their usual insulin or tablets. The sural nerve was exposed at the ankle under 2% lignocaine and the temperature recorded using a microthermocouple with a probe head diameter of 0·4 mm. Theatre temperature was constant at 21°C. The surgeon, using an operating microscope throughout, moved aside the epineurium, incised the perineurium of a superficial fascicle, and inserted the tip of an electrode into the endoneurium. The electrode was polarised to −736 mV against a standard disposable electrocardiogram electrode applied close to the wound. The flow of current was recorded when stable. A very high current (as judged from the initial calibration) suggested an air leak down the electrode track and the electrode was repositioned. Readings were

TABLE I—*Clinical details of subjects studied*

Subject	Sex and age (years)	Duration of diabetes (years)	Duration of diabetic neuropathy (years)	Vibration perception threshold (normal <20 on toe)	Thermal discrimination threshold (normal <2°C on foot)	Peroneal motor nerve conduction velocity (normal 48 (SD4) m/s)	Neuropathy grade
			Neuropathic patients				
1	M 48	16	6 months	25	>20	25·8	Severe
2	M 69	11	12	>50	>20	U/A*	Severe
3	M 51	21	2	42	>20	34·0	Moderate/severe
4	F 52	5	1	16	>20	45·0	Mild
5	M 75	5	1	12	3·2	31·7	Moderate/severe
6	M 73	21	9	>50	1·0	32·8	Moderate/severe
7	M 63	1	1	26	0·5	40·1	Moderate
8	M 36	1	1	6	2·5	41·0	Mild
9	M 67	2	10	42	>20	U/A	Severe
10	M 34	14	11 months	38	>20	U/A	Moderate
11	M 48	13	6	20	0·9	31·8	Moderate/severe
			Non-neuropathic subjects				
12	M 30	—	—	8	0·5	53·1	—
13	M 34	—	—	9	0·5	45·1	—
14	M 50	—	—	9	0·75	48·5	—
15	M 41	6 months	—	7	0·5	45·6	—

*U/A = Too low to ascertain.

TABLE II—*Endoneurial and venous oxygen tensions (mm Hg)*

Subject	Mean (SD) endoneurial oxygen	Venous oxygen	Nerve-vein gradient
	Neuropathic patients		
1	32·5 (2·1)	30	2·5
2	43·0 (only reading)	53	−10·0
3	35·5 (3·6)	41	−5·5
4	48·2 (7·6)	61	−12·8
5	45·0 (9·4)	—	—
6	29·1 (10·0)	31	−1·9
7	45·5 (18·2)	67	−21·5
8	35·2 (6·0)	48	−12·8
9	34·2 (2·0)	44	−9·8
10	51·2 (7·4)	54	−2·8
11	37·5 (7·1)	55	−17·5
	Non-neuropathic subjects		
12	53·0 (9·0)	32	21·0
13	57·0 (3·3)	30	27·0
14	46·0 (5·5)	38	8·0
15	52·2 (7·4)	50	2·2

made with eight different electrodes over a 1 cm length of nerve and from free blood around the nerve to detect false readings due to contamination. The entire procedure lasted 60-90 minutes and concluded with biopsy of the sural nerve. The wound was closed in two layers and stitches removed at 10 days.

Results

Twelve diabetic patients with neuropathy were examined. In addition, we examined three non-diabetics (three of us, who did not have biopsy) and one diabetic free of complications. Table I gives the characteristics of all these subjects. Severity of neuropathy was graded as mild, moderate, or severe in a blind fashion by two independent assessors (AJMB and JDW) taking into account symptoms, signs, and neurophysiological findings. There was agreement in seven cases and disagreement whether neuropathy was moderate or severe in four.

Table II shows the endoneurial and venous oxygen tensions recorded. In one patient the sural nerve was too fibrous for electrode insertion, and in another venous oxygen tension was not obtained. In nine out of 10 patients with both sets of readings the mean endoneurial oxygen tension was below that of the corresponding venous value. This was significantly different from the four non-neuropathic subjects, who had endoneurial oxygen tensions greater than venous (Fisher's exact test, $p<0{\cdot}02$). Comparison of medians of nerve oxygen readings from the patients and non-neuropathic subjects showed the latter to be significantly greater (unpaired Wilcoxon test, two tailed, $p<0{\cdot}05$). The nerve-venous oxygen gradient differed significantly between the two groups (unpaired Wilcoxon test, two tailed, $p<0{\cdot}05$), being reversed in the neuropathic group.

Discussion

We have described a technique which for the first time makes it possible to measure oxygen tension within human diabetic nerve in vivo. This will allow further investigation into the aetiology of diabetic neuropathy and should help to clarify the role of haematological and small vessel abnormalities in its development.

It seems probable that metabolic abnormalities within nerve have aetiological importance, but these fail to explain adequately both the development and progression of severe neuropathy in patients with apparently well controlled disease. We have confirmed in man the observation by Tuck *et al* in rats[7] that nerve in patients with neuropathy is hypoxic. We have shown an unphysiological oxygenation gradient between diabetic neuropathic nerve and venous blood from the same region, which can only worsen the metabolic environment of the nerve. This reversed gradient is not surprising if nerve blood flow in human diabetes is low, as has been shown in the rat model.[7]

Endoneurial hypoxia may arise from at least three different mechanisms. Firstly, small vessel occlusion or impaired diffusion might lead to ischaemia and hypoxia within the nerve, and preliminary examination of the biopsy specimens showed extensive endoneurial capillary disease. Secondly, diabetic nerve may consume more oxygen than normal, but this seems highly unlikely given its reduced level of activity. Thirdly, a "steal" effect may possibly have a role, in which arteriovenous shunting, well described in the foot,[10] is also operating around peripheral nerve and reducing effective circulation to the endoneurium. It remains to be determined whether endoneurial hypoxia is associated with the neuropathy or the diabetes. Further study is proceeding to examine more non-diabetics and matched non-neuropathic diabetic controls to address this question.

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